Proc. Natl. Acad. Sci. USA
Vol. 90, pp. 6844–6848, July 1993
Biochemistry

cdc2-like kinase from rat spinal cord specifically phosphorylates KSPXK motifs in neurofilament proteins: Isolation and characterization

K. TARANATH SHETTY, WILLIAM T. LINK, AND HARISH C. PANT*

Laboratory of Neurochemistry, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, MD 20892

Communicated by Thomas S. Reese, April 5, 1993

ABSTRACT **A protein kinase that phosphorylates a specific KSP sequence [K(S/T)PXK], which is abundant in high molecular weight neurofilament (NF) proteins, was identified and isolated from rat spinal cord. Characterization of this enzyme activity revealed a close relationship with p34^{cdc2} kinase with respect to its molecular mass (32.5 kDa by SDS/PAGE) and substrate specificities. It could phosphorylate a synthetic peptide analog of the simian virus 40 large tumor antigen, reportedly a specific substrate for p34^{cdc2} kinase. Histone (H1) and peptide analogs of the KSP sequence present in the C-terminal end of rat and mouse neurofilament proteins were phosphorylated. This kinase did not phosphorylate α-casein and peptide substrates of other known second messenger-dependent or -independent kinases. Dephosphorylated rat NF protein NF-H was strongly phosphorylated by the purified enzyme; NF proteins NF-M and native NF-H, but not NF-L, were slightly phosphorylated. Studies on synthetic peptide analogs of KSP repeats with substitution of specific residues, known to be present in the C-terminal regions of NF-H, revealed a consensus sequence of X(S/T)PXK, characteristic of the p34^{cdc2} kinase substrate. On Western blots, the enzyme was immunoreactive with antibody against the C-terminal end of cdc2 kinase (mouse) and neuronal cdc2-like kinase from rat but not with an antibody against the conserved PSTAIRE region of the p34^{cdc2} kinase. The antibody against the C-terminal end of cdc2 kinase could immunoprecipitate (immunodeplete) the purified kinase activity. Since the adult nervous system is composed primarily of postmitotic cells, the present observations indicate a nonmitotic role for this cdc2-like kinase activity. The effective phosphorylation of NF-H by this kinase suggests a function in axonal structure.**

Neurofilament (NF) proteins, NF-H and NF-M, contain C-terminal tail regions rich in KSP sequences (1, 2) characterized by two pentapeptide motifs, KSPXK and KSPXX. Phosphorylation of these sequences in the C-terminal domains of NF-H and NF-M is thought to affect their interactions with other components of the cytoskeletal system (3, 4). Abnormal phosphorylation of cytoskeletal proteins has been reported in several neurodegenerative disorders (5–7), and several studies have characterized kinases responsible for such phosphorylation, including those in NFs (8–10). Earlier studies established the ability of specific kinases to phosphorylate sites on the head domain of NF protein (10). In contrast, the identity of enzymes in the nervous system that could phosphorylate the specific KSP sequences in the C-terminal domains of NF proteins still remains unknown. Recently, several workers have demonstrated the phosphorylation of the C-terminal tail region of bovine NF-H by p34^{cdc2} kinase from starfish oocytes (11) and mouse mammary carcinoma (12). In view of the down-regulation of p34^{cdc2} kinase mRNA levels during the terminal differentiation of neurons (13), demonstration of NF-H phosphorylation by p34^{cdc2} kinase from nonneuronal sources, while intriguing, has uncertain physiologic relevance. The presence of consensus sequences of substrate for p34^{cdc2} kinase on vertebrate NF-H suggests the possibility that there may be cdc2-related kinases in the nervous system that phosphorylate these motifs.

In this report, we present the purification, biochemical, and immunologic characterization of a p34^{cdc2}-like kinase isolated from rat spinal cords. We demonstrate that dephosphorylated NF-H and NF-M, and synthetic peptides containing KSPXK sequences are highly effective substrates and suggest that NF protein may be one of the endogenous substrates for this cdc2-like kinase.

MATERIALS AND METHODS

Materials. Synthetic peptide analogs of KSP sequences were custom-synthesized by Peptide Technologies (Washington, DC). Peptide substrates of protein kinase A (kemptide) and S6 kinase (S6 substrate) were obtained from Peninsula Laboratories, protein kinase C, acetyl-myelin basic protein-(4–14), tyrosine kinase (RR-SRC), p34^{cdc2} kinase [21-mer peptide analog, simian virus 40 (SV40) large tumor (T) antigen], and microcystin LR were obtained from GIBCO/BRL. α-Casein and dephosphorylated casein were obtained from Sigma; histone (H1), alkaline phosphatase (calf intestine), and phosphatase-labeled goat anti-rabbit IgG were obtained from Boehringer Mannheim. Antibodies directed against C-terminal (mouse) and PSTAIRE regions (human) of p34^{cdc2} kinase were purchased from Upstate Biotechnology (Lake Placid, NY).

Extraction. Rat spinal cords (120 g, obtained from Pel-Freez Biologicals) were suspended in isotonic low-salt buffer [20 mM Tris·HCl/1 mM EDTA/1 mM EGTA/1 mM dithiothreitol (DTT)/leupeptin (5 μg/ml)/0.3 M sucrose at pH 7.5] maintained at 4°C. After removing the blood contaminants and meninges, the tissues were diced and homogenized, 1:5 (wt/vol), in high-salt extraction buffer (where 0.8 M KCl was substituted for sucrose) with a Polytron homogenizer (Brinkmann). During homogenization, 0.5 M phenylmethylsulfonyl fluoride in ethanol was added to a final concentration of 2.5 mM, and the preparation was stirred for 3 hr at 4°C. The homogenate was centrifuged (100,000 × *g* for 60 min), and the supernate was dialyzed against column-equilibrating buffer [CEB = 20 mM Tris·HCl/1 mM EDTA/1 mM EGTA/1 mM DTT/5% (vol/vol) glycerol, pH 7.5]. The dialysate was centrifuged 50,000 × *g* for 30 min, and the resulting supernate was used for further purification.

Purification. The dialyzed supernate was applied to a P-11 phosphocellulose column, equilibrated in CEB, and washed

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Abbreviations: SV40, simian virus 40; T, tumor; DTT, dithiothreitol; NF, neurofilament.

*To whom reprint requests should be addressed.

with two column volumes. Bound protein was eluted with a linear gradient of 0–2 M NaCl (in CEB) at a flow rate of 0.5 ml/min. Active fractions were pooled and concentrated in Centreprep 10 (Amicon) and size-fractionated by passing through Superdex 75 columns (Pharmacia LKB, HR 10/30, two columns in tandem) equilibrated with 50 mM Tris·HCl, pH 8.2/0.1 M NaCl/1 mM EDTA/1 mM EGTA/2 mM DTT/5% glycerol at a flow rate of 0.8 ml/min.

Active fractions obtained from the sizing step were pooled, diluted 1:10 (in the above sizing buffer without NaCl), and applied, using a Superloop (Pharmacia LKB), to a Mono Q anion-exchange column (Pharmacia LKB, HR 10/10) equilibrated in the same buffer. Kinase activity was eluted using a linear gradient of 0–1 M NaCl (in the same buffer) at a flow of 1.0 ml/min over 1 hr. These fractions were pooled, diluted 1:10 with 20 mM Hepes, pH 6.7/1 mM EDTA/1 mM EGTA/2 mM DTT/5% glycerol, and applied to a Mono S cation-exchange column (Pharmacia LKB, HR 5/5) preequilibrated with the same buffer. Bound kinase activity was eluted by a linear gradient of 0–0.2 M NaCl over 1 hr at a flow rate of 0.5 ml/min.

Peaks of activity from the Mono S separation were again size-fractionated using the above system. Individual fractions were evaluated for kinase activity and analyzed on an immunoblot with antibodies against cdc2 kinase.

Kinase Activity Measurement. Kinase activity was assayed at 30°C in 0.1 M Pipes, pH 7.0/1 mM EDTA/1 mM EGTA/1 mM DTT/5 mM $MgCl_2$/2 μM microcystin. During the course of the enzyme purification, the KSP kinase activity was monitored by using a synthetic 14-mer peptide (VKSPAKEKAKSPEK, 0.2 mM), representing two repeat KSP sequences present in the C-terminal region of mouse NF (H) as a phosphoacceptor and [γ-^{32}P]ATP (0.1 mM) as the phosphodonor substrates. The phosphorylation reaction was stopped after 30 min by spotting an aliquot of the reaction mixture onto phosphocellulose paper (Whatman, P81). Phosphate incorporation was quantified by scintillation counting, after washing in 75 mM phosphoric acid and 95% ethanol. Background incorporation (in the absence of an acceptor substrate) was subtracted from the total in all cases. Peptides with different amino acid sequences containing KSP and representing different regions of NF-H from rat and mouse and protein substrates were used to determine the substrate specificity of the kinase.

SDS/PAGE and Immunoblot Analysis. Enzyme purification was monitored by SDS/PAGE (12.5% gels) essentially as described by Laemmli (14), and the protein bands were visualized by either Coomassie blue or silver staining. Immunochemical characterization of the enzyme preparation was carried out by electroblotting the gels and probing with rabbit antibodies directed against the C terminus of neuronal cdc2-like kinase (rat residues 283–291), mouse cdc2 (residues 263–297), or the conserved PSTAIRE region of the p34^{cdc2} kinase (human cdc2 residues 42–57). The bound primary antibody was detected with goat anti-rabbit IgG coupled to alkaline phosphatase and developed with naphthol phosphate.

Immunodepletion Experiments. The enzyme preparation (50 μl) was mixed with an equal volume of phosphate-buffered saline (PBS) and 20 μl of affinity-purified polyclonal antibody against either the C terminus (mouse) or the PSTAIRE region (human) of cdc2 kinase and incubated at 4°C for 2 hr with gentle mixing. Parallel control samples that did not contain any antibody were also incubated simultaneously. Complexed and free antibodies were collected by addition of 25 μl of protein A-Sepharose beads (Pharmacia) suspended in PBS, 1/1 (vol/vol), and incubated for 90 min at 4°C. Supernates were then assayed for kinase activity using the synthetic 14-mer peptide described above.

NF Protein Phosphorylation. Preparation of NF protein from rat spinal cords was essentially as described by Tokutake *et al.* (15) and dephosphorylation was as described by Carden *et al.* (4). Residual phosphatase activity that may still be present was heat-inactivated at 60°C for 15 min. Nondephosphorylated NF protein was similarly heat-treated for subsequent use in control experiments. Both dephosphorylated and nondephosphorylated NF preparations (≈1.5 mg) were suspended in 0.5 ml of kinase assay buffer containing the enzyme preparation. Phosphorylation was initiated by addition of 0.1 mM [γ-^{32}P]ATP. Aliquots were taken after various periods of incubation (0, 2, 4, and 6 hr) and mixed with an equal volume of 2× SDS/PAGE sample buffer. NF triplet proteins were electrophoretically separated on 7.5% gels by the method of Laemmli (14). Gels were stained with Coomassie blue and autoradiographed to localize phosphorylated NF protein.

RESULTS

Extraction and Purification of KSP Kinase. The presence of KSP kinase activity in the spinal cord homogenate was identified by monitoring the phosphorylation of a 14-mer synthetic peptide analog of a KSP sequence, present in mouse NF-H, corresponding to residues 839–852. Addition of the protein phosphatase inhibitor microcystin LR (2 μM) in the kinase assay enhanced the sensitivity of the method, enabling us to quantify the kinase activity even in the crude homogenate (Table 1).

Subsequent steps to isolate the KSP kinase activity are shown in Table 1. All the kinase activity was adsorbed on the P-11 phosphocellulose column and eluted in the range of 0.45–0.60 M NaCl. The active kinase fraction from the P-11 step was size-fractionated by gel filtration and eluted at an apparent molecular mass of 95 kDa. The activity was then adsorbed on a Mono Q column at pH 8.2 and eluted in the range of 0.14–0.18 M NaCl. Fractionation on Mono S re-

Table 1. Purification of cdc2-like kinase from spinal cord

Step	Protein, mg	Activity, pmol/min	Specific activity	Purification, fold	Yield, %
Homogenate	10,740	93,188	8.7	1	100
100,000 × *g* extract	1,451	38,326	26.4	3	41
Dialysate supernatant	466	27,662	59.4	6.8	30
P-11 fractions	27	20,326	753	86.6	29
Gel filtration	19	17,410	916	105	19
Mono Q	5.3	9,772	1,844	220	10.5
Mono S peak 1	—	1,426	—	—	1.5
Mono S peak 2	0.5	2,118	4,237	487	2.3
Gel filtration of peak 1	0.0065	313	48,154	5,535	0.34
Gel filtration of peak 2	0.0053	397	74,906	8,610	0.43
Repeat Mono S of gel-filtered peak 2	0.00012	58	483,333	55,556	0.062

Activity was monitored by using the mouse NF-H-(839–852) peptide (see text). Specific activity is defined as pmol of ^{32}P-labeled protein per mg of protein per min. Protein content in the last three steps was based on A_{280}. All other protein estimation was by the method of Bradford.

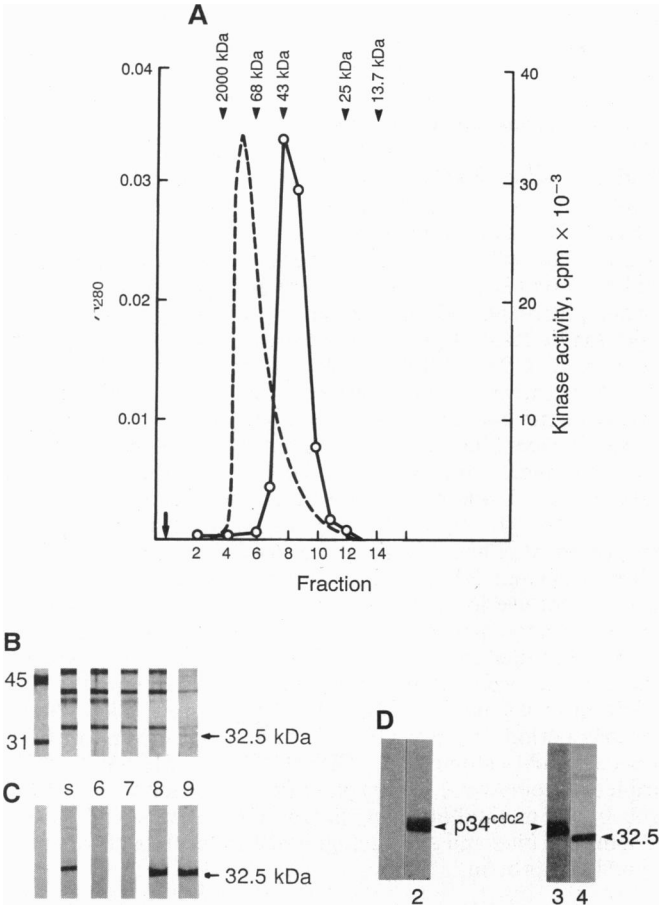

FIG. 1. (*A*) Gel filtration of Mono S peaks of kinase activity. Kinase activity with the peptide substrate KSPXK (○) is shown along with the A_{280} (dashed line) of 0.5-ml fractions. (*B*) SDS/PAGE silver stain profile of fractions from gel filtration of the Mono S peak. Lane labels represent corresponding fraction numbers. Lane S contains the initial Mono S peak sample. (*C*) Immunoblot profile of *B* with a C-terminal-region-directed antibody against $p34^{cdc2}$ kinase. (*D*) Immunoblot profiles of spinal cord-derived cdc2-like kinase (lanes 1 and 4) and authentic $p34^{cdc2}$ kinase (positive control, lanes 2 and 3) with specific polyclonal antibodies directed against $p34^{cdc2}$ kinase. Lanes 1 and 2 were incubated with antibody against the PSTAIRE region, and lanes 3 and 4 were incubated with antibody to C-terminal domains of $p34^{cdc2}$ kinase. Lanes 1 and 4 contain the cdc2-like kinase preparation from spinal cord after Mono S chromatography, and lanes 2 and 3 contain the positive controls for $p34^{cdc2}$ kinase (from epidermoid carcinoma of human A431 cell lysate). Note that immunoreactivity against C-terminal $p34^{cdc2}$ is at 34 kDa in the A431 cell lysate (lane 3), and the same antibody reacts with a protein band at 32.5 kDa in the Mono S peak 1 kinase fraction (lane 4).

solved the kinase activity into two peaks eluting at 0.08–0.09 M NaCl (peak 1) and 0.095–0.105 M NaCl (peak 2).

While there was some loss in activity at each step of purification, the greatest loss was observed during the Mono S fractionation, with only 15% recovery of the activity applied to the column. Although the kinase activity resolved at 95 kDa during the first step of size fractionation, after the Mono S step and subsequent resizing, the activity resolved as a 42-kDa peak compared to gel filtration standards (Fig. 1*A*). This suggested that some of the kinase activity had separated from the associated proteins during the Mono S fractionation. By reconstituting the fractions from the Mono S step with the active fraction, we observed a 4- to 6-fold increase in phosphorylation of the 14-mer peptide by a fraction eluting at 0.15–0.18 M NaCl. This fraction contained a single band of protein of 62 kDa by SDS/PAGE (data not shown).

After gel filtration, both peaks 1 and 2 showed identical kinase activity profiles, eluting at 42 kDa (only shown for peak 1, Fig. 1*A*). SDS/PAGE and silver staining of the active fractions revealed a protein band corresponding to 32.5 kDa, with staining proportionate to kinase activity (Fig. 1*B*). On Western blots, the antibodies against the C-terminal region of mouse $p34^{cdc2}$ kinase and rat neuronal cdc2-like kinase (data not shown) were strongly immunoreactive with the 32.5-kDa spinal cord-derived protein (Fig. 1 *C* and *D*). In contrast, the antibody to the PSTAIRE region of cdc2 was not immunoreactive with this 32.5-kDa protein band (Fig. 1*D*, lane 1). Both the PSTAIRE and the C-terminal domain antibodies against $p34^{cdc2}$ kinase strongly reacted with a band of 34 kDa in the positive control strip (consisting of human epidermoid carcinoma A431 cell lysate; Fig. 1*D*, lanes 2 and 3).

Immunoprecipitation experiments with the above antibodies showed that the antibodies against the C terminus of cdc2 (mouse) could immunodeplete the kinase activity by 95%, whereas immunoprecipitation with anti-PSTAIRE antibodies yielded only 7% immunodepletion from purified enzyme preparations. This demonstrates that the band seen on the Western blot, immunoreactive with the anti-C-terminal antibody (Fig. 1 *C* and *D*), was responsible for the cdc2-like kinase activity we observed.

Characterization of Kinase Substrate Specificity Using KSP Peptides. The kinase preparation from spinal cord could phosphorylate histone (H1) and peptide analogs of NF-H from mouse (14-mer, residues 839–852), rat (35-mer, residues 507–541), and SV40 large T antigen (21-mer, residues 118–138), a specific substrate for $p34^{cdc2}$ kinase. Substrates of second messenger-dependent and -independent kinases were not phosphorylated by these kinase preparations (Table 2). Synthetic peptide analogs of KSP repeats present in the C-terminal region of NF-H could be phosphorylated only in

Table 2. Substrate affinities for cdc2-like kinase from spinal cord

Substrate	Amino acid residues	Kinase activity			
		Peak 1		Peak 2	
		Phos.	K_m, μM	Phos.	K_m, μM
SV40 large T antigen (21-mer)	118–138	+	134	+	90
Mouse NF-H (14-mer)	839–852	+	348	+	474
Rat NF-H (35-mer)	507–541	+	—	+	—
Mouse NF-H (15-mer)	604–618	−	—	−	—
MBP (11-mer)	4–14	−	—	−	—
S_6 substrate		−	—	−	—
Tyrosine kinase substrate		−	—	−	—
Kemptide		−	—	−	—
Histone (H1)		+	21	+	19
α-Casein		−	—	−	—
Dephosphorylated casein		−	—	−	—

Phos., phosphorylation; +, phosphorylated in our assay; −, not phosphorylated in our assay. The apparent K_m is indicated. MBP, myelin basic protein.

Table 3. Substrate consensus sequence of cdc2-like kinase from spinal cord

Peptide substrate	Sequence	Phosphorylation
SV40 large T antigen-(118–138)	ADAGH**ATPPK**KKRKVEDPKDF	+
Mouse NF-H-(839–852)	**VKSPAK**EKAKSPEK	+
Rat NF-H-(814–821)	**AKSPEK**AK	+
Rat NF-H-(507–540), 35-mer	**KSPVK**EEA(KSPAEA)$_3$KSPAEVKSPA	+
Rat NF-H-(507–540), with deletion	**KSPVK**EA(KSPAEA)$_3$KSPAEVKSP	+
Rat NF-H-(507–540), with substitution	KSPVEEEA(KSPAEA)$_3$KSPAEVKSPA	−
Mouse NF-H-(513–527)	TKSRVKEEAKSPGEA	−

Boldface type indicates consensus sequence X(S/T)PXK. +, Phosphorylation; −, no phosphorylation.

the KSPXK motifs (Table 3). There was no activity toward analogs in which the proline and lysine C-terminal to serine were substituted. The data presented in Table 3 demonstrate that the consensus substrate sequence X(S/T)PXK for cdc2-like kinase from rat spinal cord is similar to that for p34^{cdc2} kinase.

Phosphorylation of NF Proteins. Alkaline phosphatase treatment dephosphorylated NF-H as indicated by the shift in electrophoretic mobility during SDS/PAGE (Fig. 2*A*, lanes 3, 6, 9, and 12). Either kinase activity (peaks 1 or 2) could phosphorylate the dephosphorylated NF-H component of the NF triplet (Fig. 2*B*). With time there was heavy labeling of dephosphorylated NF-H and slight labeling of the native NF-H after a longer incubation. A slight labeling of protein bands in the molecular mass range of NF-M was also seen, and NF-L showed no apparent phosphorylation by this kinase preparation. Under these conditions there was no apparent kinase autophosphorylation (Fig. 2, lanes 1, 4, 7, and 10).

DISCUSSION

The C-terminal part of NF-H and NF-M has an unique primary structure characterized by the highly phosphorylated repeat sequences of KSP (2). The nature of the kinases phosphorylating these KSP sequences is not known; however, Hisanaga *et al.* (11) have shown that cdc2 kinase from starfish oocytes can phosphorylate some of them. Although there have been several studies describing the nature of the kinase(s) able to phosphorylate the KSP sequences of NFs (11, 12), to date, no one has identified such an enzyme from the nervous system.

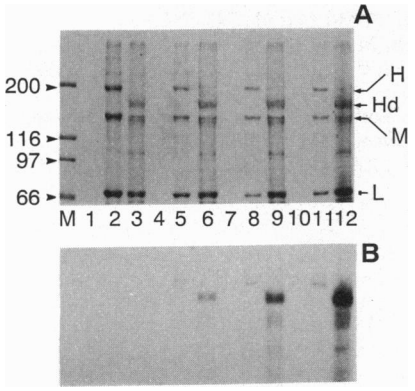

FIG. 2. Time course of phosphorylation of NF proteins by purified cdc2-like kinase from rat spinal cord. Both nondephosphorylated (native) and dephosphorylated NF preparations were used. (*A*) Coomassie blue-stained 7.5% gel. Lane M contains molecular mass markers identified to the left in kDa. Lanes 1, 4, 7, and 10 represent control (no substrate) experiments containing only kinase and [^{32}P]ATP for 0, 2, 4, and 6 hr, respectively. Lanes 2, 5, 8, and 11 represent the same time points with the addition of nondephosphorylated NFs. Lanes 3, 6, 9, and 12 represent the same time points with the addition of dephosphorylated NFs. (*B*) Autoradiographic profiles of the lanes in *A*. H, M, and L represent NF-H, NF-M, and NF-L, respectively. Hd represents the dephosphorylated NF-H. Note the extensive phosphorylation of NF-H in the dephosphorylated NF preparation in a time-dependent fashion but no effect on NF-L (lanes 3, 6, 9, and 12). In addition, note the slight phosphorylation of NF-H in the nondephosphorylated preparation (lanes 2, 5, 8, and 11).

To search for such an enzyme in the nervous system that could phosphorylate the KSP sequences in NF-H, we designed peptide substrates based on these KSP sequences and used them to monitor the enzyme activity during the purification procedure. Overall purification was >55,000-fold with a final specific activity of 483 nmol per mg per min (Table 1). Although SDS/PAGE analyses of the enzyme preparation revealed additional bands other than the 32.5-kDa cdc2-like kinase, we believe that the latter kinase is responsible for the phosphorylation of the KSPXK motif in peptides and proteins for the following two reasons: (*i*) preferred substrates for known alternative kinases were not phosphorylated by the kinase preparation and (*ii*) immunoprecipitation experiments showing that the active kinase was immunodepleted from the kinase preparation by using an antibody directed to the C-terminal region of cdc2 kinase. Since the antibodies against the PSTAIRE epitope did not immunoreact on Western blots or immunodeplete this activity, we refer to this kinase as cdc2-like kinase.

Functional characterization of the kinase revealed a substrate specificity characteristic of p34^{cdc2} with a substrate consensus sequence of XSPXK (Tables 2 and 3). cdc2-like kinase from spinal cord phosphorylated the dephosphorylated NF-H extensively and NF-M to a much lesser extent but had no effect on NF-L. These findings are consistent with the relative levels of KSPXK motifs found on these rat NF subunits: 13 on NF-H, 1 on NF-M, and none on NF-L (16). The slight phosphorylation of nondephosphorylated NF-H by the cdc2-like kinase could be due to the presence of nonphosphorylated KSPXK sites in the NF preparation.

Although dephosphorylated NF-H was extensively phosphorylated by the kinase preparation, the effect on electrophoretic mobility was small compared to the shift in mobility reported for dephosphorylated bovine NF-H after phosphorylation by cdc2 kinase from starfish oocytes (11). This difference in the magnitude of the change in electrophoretic mobility may be due to species differences with respect to the source of NF-H, to its sensitivity to phosphatase treatment, and to the kinase preparations. In this context, it is relevant to note the possible difference in the number of X(S/T)PXK epitopes present in rat and bovine NF-H. Based on cDNA sequence analysis, it is now evident that the number of consensus sequences of cdc2 kinase substrate present in NF-H differs greatly among species (refs. 17–20 and Table 4). It appears that in the rat there are very few consensus sequences for cdc2-like kinase [X(S/T)PXK] compared to X(S/T)PXX; therefore, a smaller change in mobility is to be expected after rephosphorylation. Since the cdc2-like kinase failed to phosphorylate the X(S/T)PXX motif, it is possible that there could be another variant of cdc2-like kinases, or some other kinase altogether, responsible for phosphorylation of these sites.

Table 4. Distribution pattern of X(S/T)PXK and related motifs in NF-H of various species

Sequence	Number of repeats			
	Mouse	Rat	Rabbit	Human
X(S/T)PXX	53	55	58	43
K(S/T)PXX	39	40	56	8
X(S/T)PXK	10	13	42	35
K(S/T)PXK	9	11	42	34

$p34^{cdc2}$ protein kinase is known to play a pivotal role in the eukaryotic cell cycle and has been shown to have catalytic and regulatory subunits called $p34^{cdc2}$ and cyclins, respectively (21). cDNA sequence analyses (16, 22) have revealed the existence of cdc2 variants with regard to the PSTAIRE region and appears to be tissue-specific, including in the nervous system. cdc2-like kinase from rat spinal cord, described here, showed strong immunoreactivity with antibodies against the C terminus of both mouse $p34^{cdc2}$ and neuronal cdc2-like kinase from rat. Furthermore, the observed lack of immunoreactivity with anti-PSTAIRE antibody clearly indicates this kinase differs from $p34^{cdc2}$. Recently, Lew *et al.* (23) also reported a NF kinase from bovine brain with exactly the same DNA sequence as that of the neuronal cdc2-like kinase from rat (16). Furthermore, Xiong *et al.* (24) have shown that the same kinase with a PSSLARE sequence binds to cyclin D, which they renamed as CDK5. The antibody directed against the neuronal cdc2-like kinase was generated against the identical sequence used by Xiong *et al.* (24) for CDK5. Based on the immunoreactivity profile of our enzyme preparation, as determined by antibodies directed against C-terminal cdc2 kinase (mouse) and neuronal cdc2-like kinase, it appears to be similar to CDK5.

The function of cdc2-like kinase in the nervous system, a terminally differentiated tissue, is still not very clear. The observation that the consensus sequence of the $p34^{cdc2}$ kinase substrate is present in cytoskeletal proteins, NF-H in particular, suggests it is likely that the cdc2-like kinase may be responsible for the *in vivo* phosphorylation of the C-terminal domains of NF-H and NF-M. While this phosphorylation is believed to affect their interaction with other components of the cytoskeletal system, cdc2-like kinase in the nervous system may have a regulatory role in the structural organization of the neuron.

We gratefully acknowledge the generous gift of antibody against neuronal cdc2-like kinase from Dr. Mark R. Hellmich. We are also grateful to Drs. Harold Gainer and R. Wayne Albers for their interest and helpful suggestions regarding this work.

1. Napolitano, E. W., Chin, S. S. M., Colman, D. R. & Liem, R. K. H. (1987) *J. Neurosci.* **7**, 2590–2599.
2. Shaw, G. (1991) in *The Neuronal Cytoskeleton*, ed. Burgoyne, R. D. (Wiley–Liss, New York), pp. 185–214.
3. Minami, Y. & Sakai, H. (1985) *FEBS Lett.* **185**, 239–242.
4. Carden, M. J., Trojanowski, J. Q., Schlaepfer, W. W. & Lee, V. M. Y. (1987) *J. Neurosci.* **7**, 3489–3504.
5. Sternberger, N. H., Sternberger, L. A. & Ulrich, J. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 4274–4276.
6. Goldman, J. E. & Yen, S. H. (1986) *Ann. Neurol.* **19**, 209–223.
7. Lee, L. M.-Y., Otvos, L., Jr., Schmidt, M. L. & Trojanowski, J. Q. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 7384–7388.
8. Seger, R., Ahn, N. G., Boulton, T. G., Yancopoulos, G. D., Panayotatos, N., Radziejewska, E., Ericsson, L., Bratlien, R. L., Cobb, M. H. & Krebs, E. G. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 6142–6146.
9. Wible, B. A., Smith, K. E. & Angelides, K. J. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 720–724.
10. Sihag, R. K. & Nixon, R. A. (1990) *J. Biol. Chem.* **265**, 4166–4171.
11. Hisanaga, S., Kusubata, M., Okumara, E. & Kishimoto, T. (1991) *J. Biol. Chem.* **266**, 21798–21803.
12. Guan, R. J., Hall, F. L. & Cohlberg, J. A. (1992) *J. Neurochem.* **58**, 1365–1371.
13. Hayes, T. E., Valtz, N. L. M. & McKay, R. D. G. (1991) *New Biol.* **3**, 259–269.
14. Laemmli, U. K. (1970) *Nature (London)* **277**, 680–685.
15. Tokutake, S., Hutchinson, S. B., Patcher, J. S. & Liem, R. K. H. (1983) *Anal. Biochem.* **135**, 102–105.
16. Hellmich, M. R., Pant, H. C., Wada, E. & Battey, J. F. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 10867–10871.
17. Chin, S. S. M. & Liem, R. K. H. (1990) *J. Neurosci.* **10**, 3714–3726.
18. Shneidman, P. S., Carden, M. J., Lees, J. F. & Lazzarini, R. A. (1988) *Mol. Brain Res.* **4**, 217–231.
19. Soppet, D. R., Beasley, L. L. & Willard, M. B. (1992) *J. Biol. Chem.* **267**, 17354–17361.
20. Lees, J. F., Shneidman, P. S., Skuntz, S. F., Carden, M. J. & Lazzarini, R. A. (1988) *EMBO J.* **7**, 1947–1955.
21. Lewin, B. (1990) *Cell* **61**, 743–752.
22. Meyerson, M., Enders, G. H., Wu, C.-L., Su, L.-K., Gorka, C., Nelson, C., Harlow, E. & Tsai, L.-H. (1992) *EMBO J.* **11**, 2909–2917.
23. Lew, J., Winkfein, R. J., Paudel, H. K. & Wang, J. H. (1992) *J. Biol. Chem.* **267**, 25922–25926.
24. Xiong, Y., Zhang, H. & Beach, D. (1992) *Cell* **71**, 505–514.